

Allergenicity of grass pollen from polluted and non-polluted areas measured by skin prick test and basophil activation test

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Pollution has been associated with pulmonary diseases including asthma. The bronchial inflammation caused by the contaminants increases the effect of airborne allergens in susceptible patients [1].

Asthmatic patients allergic to pollen who reside in cities with high levels of industrial pollution have a worse disease progression and more exacerbations than patients who live in cities with lower levels of pollution [2,3]. Furthermore, industrial pollution has been linked to high enterobacteria counts in grass pollen and increase in the release of endotoxins amplifying the immune response and inflammation of the airways when pollen is inhaled [4].

Furthermore, atmospheric pollution may affect plants and their pollen. Several studies have found an increase in the allergenicity of pollen from plants and trees exposed to different contaminants. However, most studies have been performed under laboratory conditions and few studies have focused on grass pollen [5-10].

Previous studies by our group have shown that pollution is associated with a reduction in photosynthetic efficacy and a higher degree of oxidative stress in grass plants and pollen proteins [8].

In the present study, we analyze the allergenicity of grass pollen from a large city with high levels of environmental pollution (Madrid) and a city with low levels of pollution (Ciudad Real) under conditions of real environmental exposure of the plants in their habitat and not in a laboratory setting.

The study was approved by the Ethics and Research Committees of the participating hospitals. All patients gave their written informed consent to participate.

Statistical analysis was performed by the SPSS software package. For the comparison of the means of quantitative variables the Student-t test was used when the data followed a normal distribution and Mann-Whitney test was used when data didn't follow it.

Madrid and Ciudad Real are located in the center of Spain. Both have a dry continental climate with short periods of grass pollination about 6 weeks in May and June.

Levels of contaminants and weather data were obtained from the City Council of Madrid and Regional Government of Castilla la Mancha websites. Data were collected from

October to May for the years 2017, 2018 and 2019 as this is the period which affects grass pollen formation.

After data analysis we found significantly higher levels of NO₂ and SO₂ in Madrid. There were no significant differences in temperature nor precipitation between Madrid and Ciudad Real (Online Supplemental files).

With regard to pollen counts, from 1st May to 30th June, daily pollen concentrations were measured in Madrid (Hospital Infanta Leonor) and Ciudad Real (Hospital General de Ciudad Real) using a Burkard spore-trap (Burkard Manufacturing Co., Rickmansworth, Herts, UK) as described before [2,3]. No significant differences in grass pollen counts were found between both cities (Online Supplemental files).

During the pollination period (May) of the years 2017, 2018 and 2019 *Lolium perenne* plants were collected in Madrid and Ciudad Real from areas near roads with vehicle traffic. The mature pollen was extracted by suction as described before [8].

Skin prick tests were performed on a sample of 75 patients with allergic rhinitis or asthma and sensitization to grass pollen, outside the grass pollen season using each original extract from the pollen collected in Madrid and Ciudad Real. 53,3% of the patients were female and the median age was 26 years. The mean wheal diameter was significantly greater with the pollen collected in Madrid (Table 1).

Basophil activation test (BAT) with both pollen extracts, expressed as the percentage of CD63+ basophils, was determined using a BASOTEST kit (Glyco-type Biotechnology GmbH, Heidelberg, Germany). The BAT was performed in 8 patients with respiratory allergy due to grass pollen. The mean percentage expression of CD63 (activated basophils) was slightly higher with the pollen from Madrid (Table 1) although the difference was not statistically significant.

In this study of grass plants collected in Madrid and Ciudad Real in their natural environment we have found that the grass pollen from Madrid produces a greater wheal size in the skin tests performed on allergic patients. Given that pollen levels, temperature and humidity are similar in both cities, we attribute this effect to the higher levels of the contaminants NO₂ and SO₂ in Madrid.

However, we have not been able to demonstrate significant differences in the BAT using both pollens, probably due to the small number of patients included in this arm of the study.

Some studies have found an increased allergenicity in pollen exposed to pollutants as CO, O₃, NO₂ and SO₂ under laboratory conditions [5-10]. However Smiljanic [9] found that pollen from *Phleum pratense* collected from highly polluted areas expressed lower levels of Phl p 6, 12 and 13 and showed with ELISA less binding to IgE in allergic patients.

Ghiani [7] conducted a study with Ambrosia pollen exposed naturally to environmental pollution, finding that pollen exposed to high levels of traffic pollution was more allergenic in SDS-PAGE and immunoblotting.

Most of these studies are based on in vitro tests with serum from patients, but García Gallardo [10] found that *Pinus radiata* pollen collected in areas of high concentrations of O₃ yielded more positive results also in skin prick tests.

In conclusion, in this study we collected pollen from grass plants exposed naturally to contaminants and analyzed the response in vivo using skin tests and, as a novel feature, also in vitro using the BAT. Skin tests revealed the greater allergenicity of the grass pollen exposed to contaminants as compared to that not exposed to such levels of pollution.

Environmental pollution constitutes an added risk factor for patients allergic to pollen, not only due to its direct proinflammatory action on the airways but also because it increases the allergenicity of the pollen itself.

Presentation in Congress

Data have been presented in electronic poster form at 33 Congreso de Sociedad Española de Alergología e Inmunología Clínica (SEAIC) in October 2021.

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Conflicts of Interests

The authors declare no conflicts of interests

References

1. Air Pollution and Climate Change Task Force of the Italian Society of Allergology, Asthma and Clinical Immunology (SIAAIC), Patella V, Florio G, Magliacane D, Giuliano A, Crivellaro MA, et al. Urban air pollution and climate change: “The Decalogue: Allergy Safe Tree” for allergic and respiratory diseases care. *Clin Mol Allergy*. 2018;16(1):20.
2. Mur Gimeno P, Feo Brito F, Martínez C, Tobías A, Suárez L, Guerra F, et al. Decompensation of pollen-induced asthma in two towns with different pollution levels in La Mancha, Spain. *Clin Exp Allergy*. 2007;37(4):558-63.
3. Feo Brito F, Mur Gimeno P, Martínez C, Tobías A, Suárez L, Guerra F, et al. Air pollution and seasonal asthma during the pollen season. A cohort study in Puertollano and Ciudad Real (Spain). *Allergy*. 2007;62(10):1152-7.
4. Alfaya T, Feo Brito F, García Rodríguez C, Pineda F, Lucas JA, Gutiérrez Mañero FJ, et al. *Lolium perenne* pollen from a polluted city shows high allergenic potency and increased associated Enterobacteriaceae counts. *J Investig Allergol Clin Immunol*.

2014;24(2):132-4.

5. Cuinica LG, Cruz A, Abreu I, da Silva JCGE. Effects of atmospheric pollutants (CO , O_3 , SO_2) on the allergenicity of *Betula pendula*, *Ostrya carpinifolia*, and *Carpinus betulus* pollen. *Int J Environ Health Res*. 2015;25(3):312-21.
6. Eckl-Dorna J, Klein B, Reichenauer TG, Niederberger V, Valenta R. Exposure of rye (*Secale cereale*) cultivars to elevated ozone levels increases the allergen content in pollen. *J Allergy Clin Immunol*. 2010;126(6):1315-7.
7. Ghiani A, Aina R, Asero R, Bellotto E, Citterio S. Ragweed pollen collected along high-traffic roads shows a higher allergenicity than pollen sampled in vegetated areas. *Allergy*. 2012;67(7):887-94.
8. Lucas JA, Gutierrez-Albanchez E, Alfaya T, Feo-Brito F, Gutiérrez-Mañero FJ. Oxidative stress in ryegrass growing under different air pollution levels and its likely effects on pollen allergenicity. *Plant Physiol Biochem*. 2019;135:331-40.
9. Smiljanic K, Prodic I, Apostolovic D, Cvetkovic A, Veljovic D, Mutic J, et al. In-depth quantitative profiling of post-translational modifications of Timothy grass pollen allergome in relation to environmental oxidative stress. *Environ Int*. 2019;126:644-58.
10. García-Gallardo MV, Algorta J, Longo N, Espinel S, Aragonés A, Lombardero M, et al. Evaluation of the Effect of Pollution and Fungal Disease on *Pinus radiata* Pollen Allergenicity. *Int Arch Allergy Immunol*. 2013;160(3):241-50.

Table 1. Skin prick tests (mean diameter: major x orthogonal diameter) and Basophil activation test (percentage of CD63+ basophils) with grass pollen extracts from Madrid and Ciudad Real.

	POLLEN A (CIUDAD REAL)	POLLEN B (MADRID)
SKIN PRICK TEST (mm) n=75 * p<0.01		
Mean	35,44	43,69
Median	30	35
Stand. Desv.	24,44	31,24
Min	4	9
Max	130	150
BASOPHIL ACTIVATION TEST (%CD63) n=8		
Mean	56,25	60,58
Median	59,35	70,7
Stand. Desv.	35,17	32,55
Min	9	13,8
Max	95,7	93